

Endothelin and aneurysmal subarachnoid haemorrhage: a study of subarachnoid cisternal cerebrospinal fluid

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Abstract

Endothelin (ET) is considered one of the most potent vasoconstrictor polypeptides; several experimental studies have suggested its possible role in the pathogenesis of arterial vasospasm after subarachnoid haemorrhage (SAH). Previously reported data on plasma and CSF levels of endothelin in patients with a diagnosis of SAH have been controversial. Cisternal endothelin CSF levels and the possibility that they could be related to vasospasm and other clinical patterns of SAH were investigated. CSF samples were obtained from 55 patients admitted after angiographic diagnosis of intracranial aneurysm. Levels of ET-1 and ET-3 were measured through radioimmunoassay technique. Twelve patients who had operations for unruptured aneurysms were considered control cases; 43 patients with SAH were classified according to: Hunt and Hess grading at admission, vasospasm grading, CT classification and timing of surgery. In all 55 patients ET-1 was measured, while positive levels of ET-3 were found only in 17 cases of 48. No linear correlation was found between cisternal CSF ET-1 levels when considering time of surgery, CT classification, Hunt and Hess grading at admission, and vasospasm grading. The results of ET-3 assay should be considered with great caution because of the low percentage of positive cases. Cisternal CSF levels of ET-1 and ET-3 are not directly related to the occurrence of arterial vasospasm after the aneurysm rupture, or to other major clinical patterns of SAH; however, ET-1 expression occurs either in paraphysiological (unruptured aneurysm) or in pathological conditions (SAH). It is suggested that ET may potentiate, or may be potentiated by, other factors playing a consistent pathophysiological role in the development of vasospasm.

Endothelin shows a significant contractile response in cerebral arteries due to a large influx of Ca^{++} through voltage-dependent Ca^{++} channels.² Much experimental work has been carried out especially on aneurysmal subarachnoid haemorrhage (SAH) and the possible involvement of ET in the pathogenesis of arterial vasospasm.³⁻⁸ Two mechanisms were identified in signal transduction in cerebral arteries.⁹ The former is specific for ET-1, depends on a specific receptor largely distributed in CNS,¹⁰ and is related to the initial vasoconstrictor effect (calmodulin-dependent mechanism). The latter is common to all three ETs and leads to the characteristic and prolonged vasoconstriction through the activation of a G protein and the protein kinase C.¹¹ Moreover, ET participates in the enhancement of cellular proliferation in tissues other than vascular endothelium, acting as a local autacoid and activating specific proto-oncogenes.^{1,12} The endothelial damage due to subarachnoid blood deposition enhances platelet adhesion and thrombus formation^{13,14} and blood clots and platelet-degradation compounds (thrombin, B-TGF) may induce the synthesis of mRNA encoding for ET and the consequent release of the vasoconstrictive peptide.

However, data available from previous studies on ET levels in the CSF and plasma of patients with SAH are controversial regarding the possible relationship with arterial vasospasm occurrence (table 1). Some authors found a direct correlation between a significant peak in plasma¹⁵ and CSF^{16,17} ET-1 levels and the occurrence of symptomatic vasospasm; in other series¹⁸ no correlation was found between CSF ET levels and vasospasm, although a higher plasma ET-1 level at 8-14 days after SAH was found and related to a possible "stress response" in patients showing clinical signs of vasospasm.¹⁸ These data do not support the theory that CSF ET levels may represent a predictive factor for clinical outcome, mainly regarding the occurrence of vasospasm.

In this study, we analysed cisternal CSF levels of ET-1 and -3 in a series of 55 patients, to verify any possible correlation with different clinical patterns of the disease: cisternal CSF is strictly in contact with the arterial branches displaying vasospasm and has been considered a more reliable mirror of neurochemical SAH-related patterns,¹⁹ than ventricular or lumbar CSF.

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Endothelin (ET) has been shown to be one of the most potent vasoconstrictor polypeptides.¹ Human genome encodes for three different subtypes which show a constrictive effect in order of ET-1 > ET-2 > ET-3.

Table 1 Summary of data available in reports on measurement of endothelin levels in cerebrospinal fluid of patients after subarachnoid haemorrhage

| Author, year | Metabolite | Source | Number of cases | Comments |
|--------------------------------|------------|--------------------|-----------------|--|
| Suzuki H, 1990 ¹⁶ | ET-1 | Cisternal drainage | 26 | Significant increase of ET-1 at day 4-6 after SAH. |
| Fujimori A, 1991 ¹⁸ | ET-1 | CSF Plasma | 25 20 | No correlation with vasospasm onset. Correlated with vasospasm only in the 2nd week after SAH (possible "stress response"). |
| Kraus GE, 1991 ²⁷ | ET-1 | Ventricular | 4 | No correlation with vasospasm. |
| | ET-3 | CSF | 4 | Significant correlation with vasospasm onset. |
| Suzuki R, 1992 ¹⁷ | ET-1 | Cisternal CSF | 28 | Increased levels of ET-1 between day 5-7 after SAH only in patients presenting with vasospasm. |

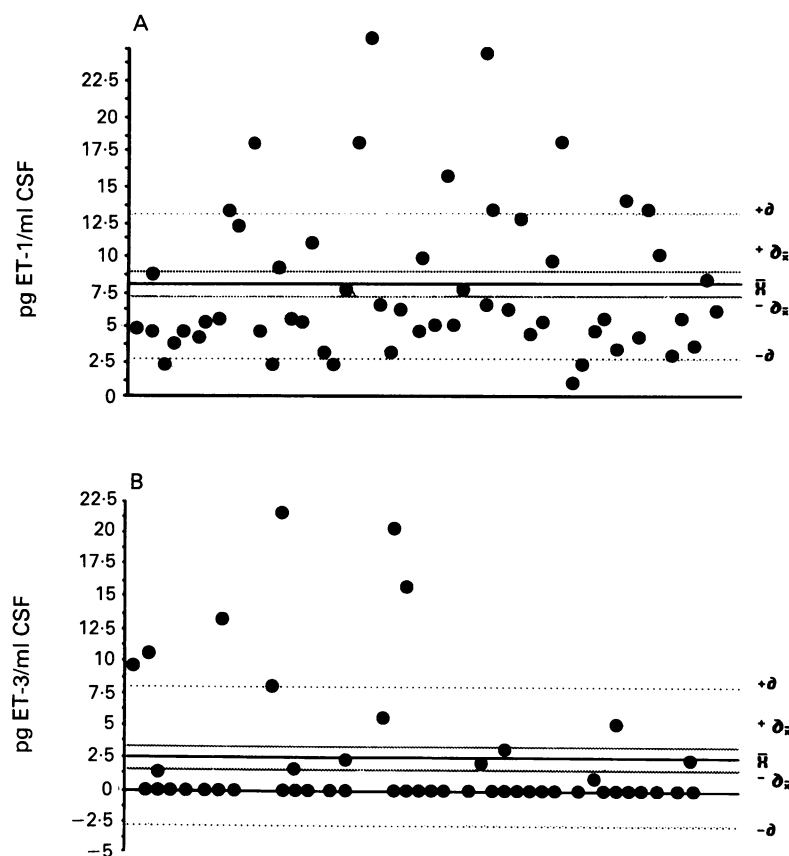


Figure 1A Scattergram of individual cases of cisternal CSF levels of ET-1 in 55 patients operated on for intracranial aneurysm. ET-1 level is expressed in pg/ml of CSF. In the scatter plot the mean (\bar{x}), SEM and SD are represented; B) Scattergram of individual cases of cisternal CSF levels of ET-3 in 47 patients operated on for intracranial aneurysm. ET-3 level is expressed in pg/ml of CSF. In the scatter plot the mean (\bar{x}), SEM and SD are represented.

Materials and methods

CLINICAL MATERIAL

We studied a series of 55 selected patients who were operated on for intracranial aneurysms. In 12 cases an unruptured intracranial aneurysm was diagnosed during angiographic studies performed for other reasons (previous episodes of transient cerebral ischaemia): these cases represent the control group, although we cannot exclude that the anomalous arterial wall of the aneurysm might alter ET-1 and ET-3 levels in the nearby cisternal CSF. For ethical reasons, however, it was not possible to obtain "pure" control cisternal CSF samples and we have to consider the above mentioned cases as the best control samples. Thus differences observed in the SAH group depend on occurrence of haemorrhage. Forty three patients

with diagnosis of aneurysmal SAH were included and classified according to different parameters: timing of surgery, CT classification at diagnosis according to Fisher *et al.*²⁰ and Hunt and Hess grading²¹ at admission.

VASOSPASM ASSESSMENT

Vasospasm was assessed by angiographic study and transcranial Doppler (TCD) serial measurements. Patients operated on day 1-4 from the last SAH episode had angiography on admission, while patients operated on after the tenth day from the last SAH episode did not have angiography before day 8 post haemorrhage. All patients were studied with serial TCD measurements every second day after SAH, to confirm if there was vasospasm during the maximum expectation time. As in previous reports^{22,23} we considered the mean velocities found in the middle cerebral artery (MCA) and the internal carotid artery (ICA) as there is a significant correlation between vasospasm intensity and flow velocity measured by TCD in these territories. Combining the data of angiographic study (according to Fisher's criteria²⁴) and TCD, the following groups were considered: vasospasm (VSP) = 0 for patients with unruptured aneurysm; VSP = 1 for minimal changes and/or mean flow velocity between 80 and 100 cm/s during the first two weeks after SAH; VSP = 2 for the anterior carotid artery (ACA) and/or MCA diameter at least 1 mm wide and/or mean flow velocity between 100 and 120 cm/s; VSP = 3 for ACA and/or MCA diameter of 0.5 mm and/or mean flow velocity between 120 and 160 cm/s; VSP = 4 for ACA and/or MCA diameter <0.5 mm and/or mean flow velocity >160 cm/s.

CSF samples were obtained during surgery by cisternal puncture of the subarachnoid cistern the nearest to the aneurysm before aneurysm exclusion. When necessary, patients undergoing early surgery were treated with osmotic agents (mannitol 20%), and clonidine. Patients undergoing delayed surgery were treated with tranexamic acid (6 g/day iv) and osmotic agents until surgery.

RADIOIMMUNOASSAY

All CSF samples were rapidly frozen in vials after centrifugation and stored at -80°C until analysis. The radioimmunoassay (RIA) was performed on the CSF after protein extraction.²⁵ ET-1 and ET-3 were measured according to the method of Kraus *et al.*²⁶ using kits from Peninsula Laboratories,

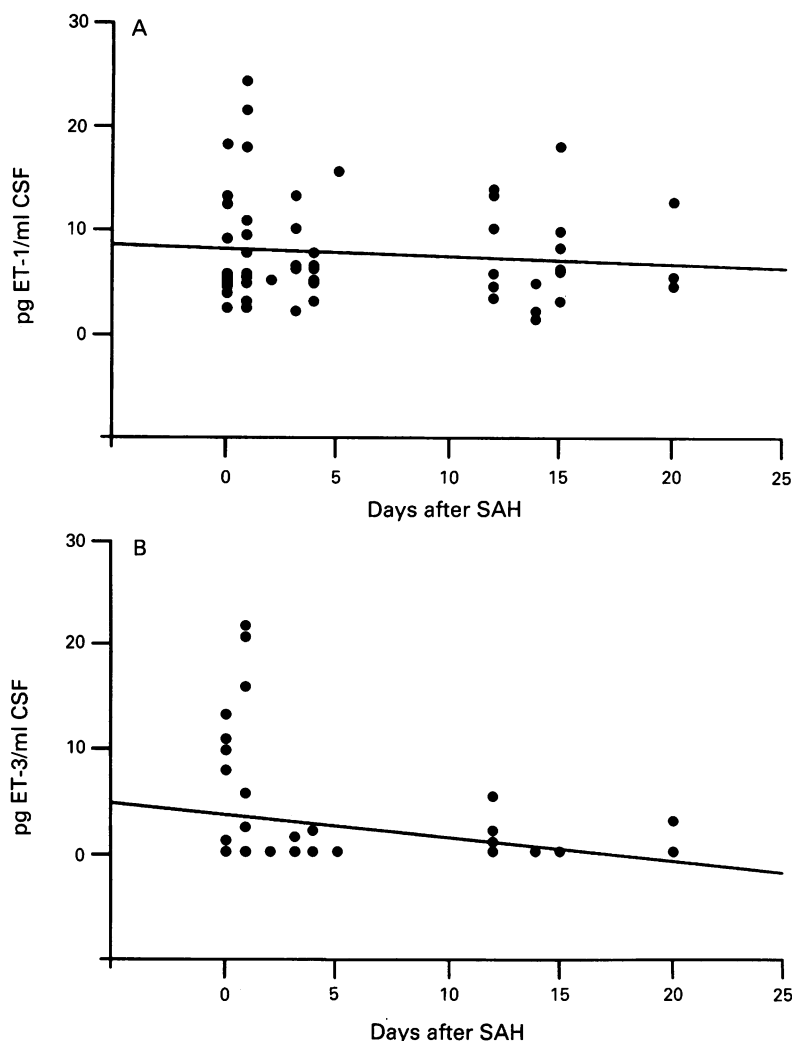


Figure 2A, B Linear regression plot showing the relationship between the time of surgery and cisternal CSF level of ET-1 (fig 2A) and ET-3 (fig 2B). There is no statistical correlation between variables (For ET-1: $R = 0.092$; F ratio 0.453; ET-3: $R = 0.317$; F ratio 5.042).

Belmont, CA. Both RIA kits are ^{125}I labelled and use rabbit antiserum specific for endothelin (^{125}I -ET-1 from pig and ^{125}I -ET-3 from rat or human), and goat antirabbit IgG serum. The ET-1 assay has a cross-reactivity of 7% with ET-2 and ET-3; the assay for ET-3 has crossreactivity <2% with ET-1 and <1% with ET-2. Results are expressed in pg/ml of CSF.

Table 2 Cisternal CSF levels of ET-1 (pg/ml of CSF) in 55 cases of intracranial aneurysm

| Clinical pattern | Number | Range | Mean (SEM) |
|------------------|--------|----------|--------------|
| CT 0 (no SAH) | 12 | 2.4-18.3 | 7.39 (1.39) |
| CT 1 | 19 | 1.2-18.1 | 7.02 (0.97) |
| CT 2 | 12 | 2.3-15.7 | 7.50 (1.32) |
| CT 3 | 12 | 2.4-24.4 | 9.85 (2.15) |
| H&H 0 (no SAH) | 12 | 2.4-18.3 | 7.39 (1.39) |
| H&H 1-1a | 19 | 1.2-13.4 | 5.72 (0.74) |
| H&H 2 | 17 | 2.4-24.4 | 9.88 (1.51) |
| H&H 3 | 7 | 3.5-21.5 | 9.29 (2.42) |
| VSP 0 (no SAH) | 12 | 2.4-18.3 | 7.39 (1.39) |
| VSP 1 | 24 | 1.2-24.4 | 7.28 (1.09) |
| VSP 2 | 9 | 3.1-18.1 | 10.14 (1.60) |
| VSP 3-4 | 10 | 2.4-21.5 | 7.55 (1.93) |

Statistical analysis (ANOVA, Student's t test with Bonferroni correction) revealed no significant differences among subgroups. The extent of subarachnoid clot at CT scan is classified according to Fisher *et al.*²⁰ clinical classification at admission according to the Hunt and Hess grading system²¹; for the classification of arterial vasospasm, see Materials and methods.

Table 3 Cisternal CSF levels of ET-3 (pg/ml of CSF) in 47 cases of intracranial aneurysm

| Clinical pattern | Number | Range | Mean (SEM) |
|------------------|--------|--------|-------------|
| CT 0 (no SAH) | 12 | 0-13.3 | 3.59 (1.5) |
| CT 1 | 17 | 0-21.7 | 1.61 (1.27) |
| CT 2 | 10 | 0-5.22 | 0.77 (0.52) |
| CT 3 | 9 | 0-20.5 | 5.19 (2.56) |
| H&H 0 (no SAH) | 12 | 0-13.3 | 3.59 (1.5) |
| H&H 1-1a | 17 | 0-21.7 | 2.16 (1.28) |
| H&H 2 | 14 | 0-20.5 | 2.77 (1.77) |
| H&H 3 | 5 | 0-5.22 | 1.23 (1.01) |
| VSP 0 (no SAH) | 12 | 0-13.3 | 3.59 (1.5) |
| VSP 1 | 21 | 0-21.7 | 3.22 (1.5) |
| VSP 2 | 7 | 0-5.6 | 0.80 (0.8) |
| VSP 3-4 | 8 | 0-5.22 | 1.07 (0.66) |

Statistical analysis (ANOVA, Student's t test with Bonferroni correction) revealed no significant differences among subgroups. The extent of subarachnoid clot at CT scan is classified according to Fisher *et al.*²⁰; clinical classification at admission according to the Hunt and Hess grading system²¹; for the classification of arterial vasospasm, see Materials and methods.

STATISTICAL ANALYSIS

To verify any possible correlation between cisternal CSF levels of ET and different clinical parameters a linear regression analysis was undertaken. Statistical evaluation for inter-group comparison was also performed using ANOVA and Student's t test for unpaired data and the Bonferroni method²⁷; statistical significance was $p < 0.05$. Statistical analysis of data regarding ET-3 was recorded considering all the negative cases to be of value 0, although the percentage of these cases was higher than that of the positive ones. However, a statistical analysis which considered only positive ET-3 values was performed separately.

Results

In all 55 patients RIA for ET-1 was performed, while only 48 CSF samples were assayed for ET-3. A positive response for ET-1 was observed in all 55 cases; the positive response for ET-3 was significantly lower (17 of 48 cases). Figures 1a and b show the scatter plots of the results of ET-1 and ET-3 assays for individual cases.

Four patients showed clinical evidence of arterial vasospasm with angiographical demonstration of high-degree arterial narrowing: for statistical reasons we grouped these cases with six patients classified with grade 3 VSP according to the grading of Fisher *et al.*²⁴ and TCD mean velocity >120 cm/s, while mild and transient neurological deficits were recorded in this subgroup.

Twelve patients were operated on for unruptured aneurysms and were classified as CT 0, Hunt and Hess 0 and vasospasm 0. Mean (SEM) ET-1 cisternal CSF level was 7.39 (1.39) (range 2.4-18.3); ET-3 was detected in 5 of 12 cases with a mean (SEM) level of 3.59 (1.5) pg/ml of CSF (range 0-13.3). Twenty four patients were operated on between day 1 and 4 from the last SAH episode: mean (SEM) cisternal CSF level of ET-1 was 8.56 (1.22) pg/ml and mean ET-3 level (17 cases: 8 positive results) was 4.12

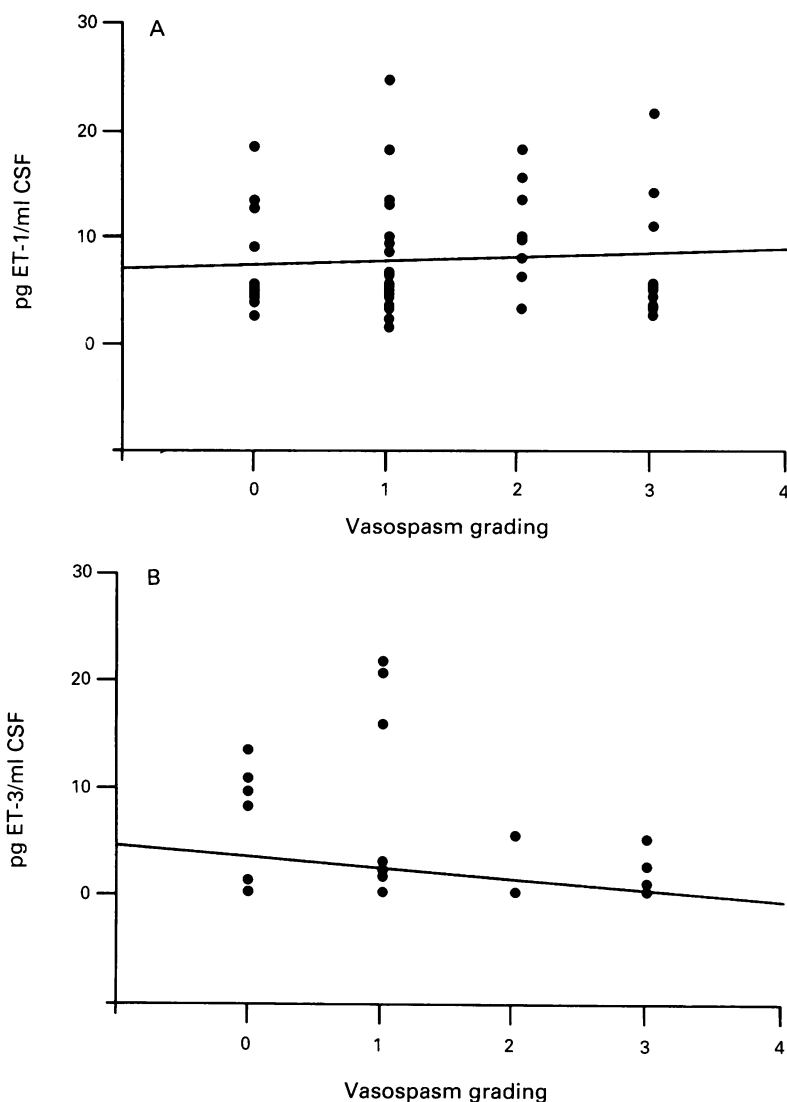


Figure 3A, B Linear regression plot showing the relationship between the vasospasm classification (see Materials and methods) and cisternal CSF level of ET-1 (fig 3A) and ET-3 (fig 3B). There is no statistical correlation between variables (For ET-1: $R = 0.072$; F ratio 0.273; ET-3 $R = 0.184$; F ratio 1.583).

(1.82) pg/ml. In 19 patients treated in the delayed surgery group, mean (SEM) ET-1 CSF level was 7.16 (1.06) pg/ml and mean (SEM) ET-3 level was 0.62 (0.32) pg/ml due to the lower number of positive responses (four out of 19 cases). Statistical analysis and in particular linear regression showed no significant correlations between both ET-1 and ET-3 CSF levels and the time of surgery (fig 2a and b).

Tables 2 and 3 report mean (SEM) of ET-1 and ET-3 in different subgroups of patients classified according to the Hunt and Hess grading on admission, CT scan and vasospasm classification. Linear regression showed that there was no significant correlation between ET-1 and ET-3 CSF levels and the clinical patterns considered (fig 3–5).

ANOVA analysis followed by Student's t test and Bonferroni correction showed no statistical differences among subgroups: however, ET-3 levels seem to be slightly higher in cisternal CSF of patients with higher degree of cisternal clot deposition

(CT 3) when compared with CT 1 and CT 2 subgroups; thereafter, the high standard deviation of the data and the relative high level of ET-3 in control cases did not enable us to consider this result definitive. No significant differences were found in ET-3 levels in different subgroups, when only the positive cases were considered.

Discussion

A considerable amount of experimental work has been published which suggests a possible role for ET-1 in the pathogenesis of arterial vasospasm following aneurysmal SAH. A summary of these data is largely available in recent reviews.^{12,26} ET-1 has a potent constrictor effect on major arteries in vivo in most of the systemic vascular beds, and causes a long-lasting pressor response when injected iv in rats. ET-3 has been shown to have similar effects.^{26,28} As for cerebral vasculature, ET-1, only when injected intracisternally, induced a long-lasting vasoconstrictor effect on the basilar artery of cats,²⁹ in adult mongrel dogs⁴ and also induced a significant reduction of regional CBF in cats³⁰ and in rats.³¹ In rats local CBF was also significantly reduced through topical application of ET-1.³² When administered into the vertebral artery, ET-1 did not cause any vasoconstrictive effect and was not distributed inside the CNS, except in areas unprotected by the blood-brain barrier.^{33,34} Moreover, histological changes shown in the arterial wall after a continuous injection of ET-1 did not significantly differ from degenerative changes observed in spastic basilar arteries of dogs after a double intracisternal injection of arterial blood.⁵

Previously reported values of ET-1 and ET-3 CSF levels after aneurysmal SAH in humans vary widely because of the different methods used in CSF sampling.^{14,16,26} For example, Fujimori *et al.*¹⁸ did not find any correlation between serial ET levels obtained in CSF collected through a catheter placed in basal cisterns or in the lateral ventricle, while Kraus *et al.*²⁶ reported higher ET-3 levels in CSF collected by ventriculostomy in a small series (four patients with symptomatic vasospasm).

The results of our study suggest that the measurements of cisternal CSF ET-3 levels should be considered with extreme caution. No linear correlation was found between cisternal CSF ET-3 levels, vasospasm grading and the Hunt and Hess clinical classification at admission. Afterwards, the low percentage of positive cases (<20%) and the wide data range suggest that, after SAH, ET-3 is rarely seen and might be considered an epiphenomenon of brain damage following the haemorrhage, as in the high levels for head injuries reported by Kraus *et al.*²⁶ Thereafter, CSF ET-3 levels are, in our series, quite low when compared with values reported by others: this might be related to the different technique used in deproteinisation and to the different source of CSF samples.

The analysis of data for ET-1, which is a

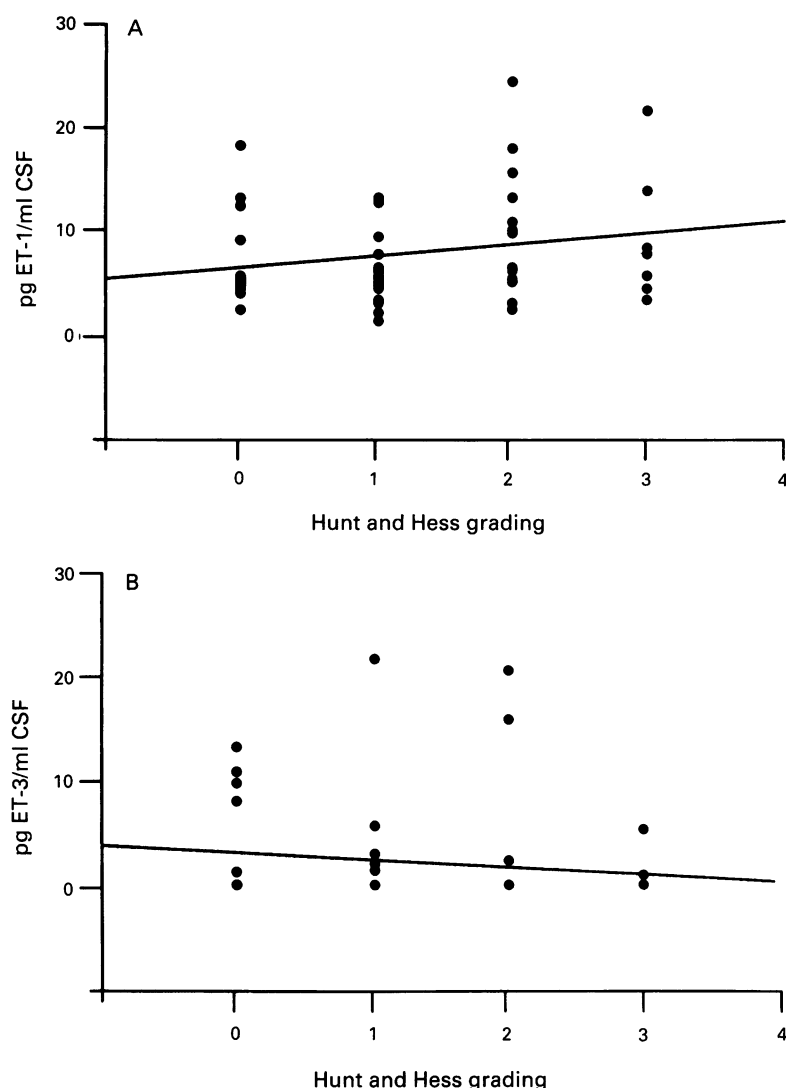


Figure 4A, B Linear regression plot showing the relationship between the Hunt and Hess grade at admission and cisternal CSF level of ET-1 (fig 4A) and ET-3 (fig 4B). There is no statistical correlation between variables (For ET-1: $R = 0.221$; F ratio 2.729; ET-3 $R = 0.099$; F ratio 0.508).

more potent vasoconstrictor agent than ET-3, raises some questions: Suzuki *et al*¹⁷ recently suggested that the higher levels of plasma ET-1 could be related to the extent of subarachnoid clots and that the increased values of the compound found in CSF of patients with arterial vasospasm during the second week after SAH may reflect the endothelial damage and the consequent breakdown of blood-brain barrier. According to these findings, we had to expect that ET-1 levels in cisternal CSF would be clearly related to CT scan classification of subarachnoid clot. In our series, in patients operated on without diagnosis of previous SAH (control cases), the expression of ET-1 is quite similar to that observed in patients with diagnosis of SAH. Moreover, there is no significant correlation between ET-1 CSF levels and the amount of subarachnoid clot as shown by CT. These results suggest that there is no close relationship between the cisternal CSF level of the polypeptide and the aneurysm bleeding.

Our data for VSP do not agree with those of other authors¹⁷: however, we considered a

TCD and angiographical classification of arterial spasm which seems to be a more reliable method than the clinical judgment used in other studies.¹⁷ Conversely, when considering the clinical classification of patients on admission (Hunt and Hess grading system), we observed a slight increase of ET-1 cisternal CSF levels in patients in grades 2 and 3 compared with control cases and to patients with minimal signs (grades 0 and 1). However, no direct correlation was found between ET-1 cisternal CSF level and clinical subgroups (fig 3a).

Thus the general conclusion from our results is that cisternal CSF levels of ET-1 and -3 are unrelated to vasospasm and to other specific clinical characteristics of aneurysmal SAH; however, some aspects may need emphasis when discussing this lack of correlation.

First, a 'normal range' of CSF ET was not available until now because of the different methods used in deproteinisation and of the different characteristics of CSF samples considered.^{18,35} ET does not penetrate through the endothelium and may act only from the adventitial site, as reported in other studies.^{12,29} The CSF sampling from the subarachnoid cisterns through a direct puncture before the aneurysm exposure and clipping procedure, enabled us to detect a level of ET-1 and ET-3 which was marginally affected by the 'stress response' due to surgical procedures^{36,37} and to avoid questionable interpretations of data obtained through long-lasting placement of a cisternal catheter in the subarachnoid space.³⁷

The second instance concerns the biological characteristics of ET which is considered as a local autacoid whose synthesis is regulated at a level of mRNA transcription and may be transiently induced by the accumulation of thrombin, B-TGF, shear stress and other factors after aneurysmal rupture.^{1,12} Thus measurements of cisternal CSF levels may not reflect events related to a local factor, such as the proliferative angiopathy characteristic of post-haemorrhagic vasospasm.

Third, ET has been demonstrated to activate the phospholipase A2 enzymatic pathway via a G protein and the consequent release of metabolites. In some series this has been correlated with symptomatic vasospasm, such as, leukotrienes¹⁹ and lipid hydroperoxides.³⁸ Thus ET might, at least partly, indirectly participate in mechanisms involved in the pathogenesis of arterial vasospasm.

Finally, we have to take into account that patients with unruptured aneurysm are, as previously discussed, the best control material and that cisternal CSF sampling seems to be a more reliable mirror of local metabolic change than lumbar or ventricular CSF.¹⁹ Our results suggest that the haemorrhagic event, in view of the different clinical patterns (and mainly vasospasm), does not influence ET-1 and ET-3 synthesis and their release in cisternal CSF. We cannot, however, exclude the possibility that the presence of the abnormal wall of the aneurysm per se may exert

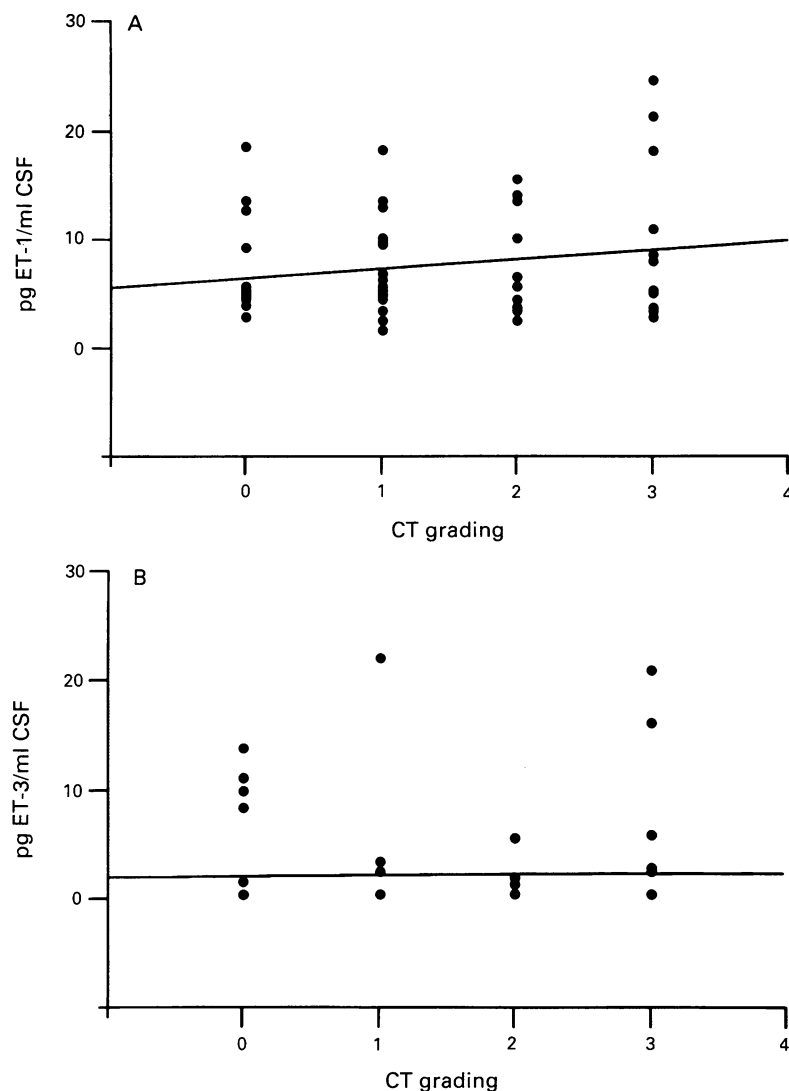


Figure 5A, B Linear regression plot showing the relationship between the CT scan classification of the extent of the subarachnoid clot and cisternal CSF level of ET-1 (fig 5A) and ET-3 (fig 5B). There is no statistical correlation between variables (For ET-1: $R = 0.165$; F ratio 1.487; ET-3: $R = 0.058$; F ratio 0.152).

some influence on cisternal CSF level of ETs. Given that there is no clear correlation between cisternal CSF ET-1 levels and time of surgery, we speculate that ET-1 may be expressed either in para-physiological or in pathological conditions, such as SAH. In some cases ET may potentiate, or may be potentiated by, other factors, such as haemoglobin derivative factors from the cisternal blood clot,³⁹ and leukotrienes or other eicosanoids from the brain compartment, that play a complementary pathophysiological role in the development of arterial vasospasm.

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